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☐ 1: Protein Expr Purif. 2000 Apr;18(3):388-93.

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ELSEVIER SCIENCE
FULL-TEXT ARTICLE

Refolding and purification of *Zymomonas mobilis* levansucrase produced as inclusion bodies in fed-batch culture of recombinant *Escherichia coli*.

PubMed
Services

Sunitha K, Chung BH, Jang KH, Song KB, Kim CH, Rhee SK.

Korea Research Institute of Bioscience and Biotechnology, Taejon, Yusong, 305-600, Korea.

Related
Resources

Zymomonas mobilis levansucrase was overproduced by the fed-batch culture of recombinant *Escherichia coli* harboring a novel expression system that is constitutively expressed by the promoter from the *Rahnella aquatilis* levansucrase gene. Most of the levansucrase was produced as inclusion bodies in the bacterial cytoplasm, accounting for approximately 20% of the total cellular protein. Refolding after complete denaturation by high concentrations of urea or guanidine hydrochloride was not successful, resulting in large amounts of insoluble aggregates. During the development of the refolding method, it was found that direct solubilization of the inclusion bodies with Triton X-100 reactivated the enzyme, with a considerable refolding efficiency. About 65% of inclusion body levansucrase was refolded into active levansucrase in the renaturation buffer containing 4% (v/v) Triton X-100. The in vitro refolded enzyme was purified to 95% purity by single-step DEAE-Sepharose ion exchange chromatography. Triton X-100 was removed by this ion exchange chromatography. Copyright 2000 Academic Press.

PMID: 10733894 [PubMed - indexed for MEDLINE]

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L1 QUE FRUCTOTRANSFERASE

FILE 'CAPLUS, SCISEARCH, BIOSIS, PASCAL, BIOTECHDS, JICST-EPLUS, LIFESCI, FSTA' ENTERED AT 09:57:12 ON 27 JUN 2003

L2 156 S L1 AND (LEVAN OR UREAFACIENS OR DIFRUCTOSE DIANYHYDRIDE)
L3 82 S L2 AND (PURIF? OR ISOLAT? OR CHARACT? OR CLON?)
L4 6 S L3 AND (MOBILIS)
L5 2 DUP REM L4 (4 DUPLICATES REMOVED)
L6 6 S L2 AND MOBILIS
L7 2 DUP REM L6 (4 DUPLICATES REMOVED)
L8 35 DUP REM L3 (47 DUPLICATES REMOVED)
L9 2 S L8 AND ZYMOMONAS

ACCESSION NUMBER: 1984:63893 CAPLUS

DOCUMENT NUMBER: 100:63893

TITLE: Action of **levan fructotransferase**
of *Arthrobacter ureafaciens* on
levan oligosaccharidesAUTHOR(S): Tanaka, Kuniiji; Karigane, Takashi; Yamaguchi, Fumio;
Nishikawa, Shigeko; Yoshida, Noriko

CORPORATE SOURCE: Dep. Biol., Osaka Kyoiku Univ., Osaka, 543, Japan

SOURCE: Journal of Biochemistry (Tokyo, Japan) (1983), 94(5),
1569-78

CODEN: JOBIAO; ISSN: 0021-924X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Levan fructotransferase (I) of *A. ureafaciens***

, which produces di-D-fructose 2,6':6,2' dianhydride (difructose anhydride IV) from **levan** by an intramol. transfructosylation reaction, was **purified** to give a single protein band of pI 4.5-4.7 on isoelec. focusing. I had a mol. wt. of 128,000 by gel filtration on Sephadex G-200 and 60,000 on SDS-polyacrylamide disc gel electrophoresis, suggesting that the enzyme is composed of 2 identical subunits. The shortest levan oligosaccharide chain required for difructose anhydride IV formation was detd. to be tetraose. TLC of the enzymic digest of a modified levanhexaose derived from levanhexaose by the redn. of the reducing end to an alditol residue with NaBH₄ gave the difructose anhydride IV spot, suggesting that I attacks the modified levanhexaose mol. from the direction of the nonreducing fructose end. Enzymic digests of levantetraose, -pentaose, and -hexaose gave, in addn. to the difructose anhydride IV spot, spots of oligofructans of lower mobility than the original substrate on TLC. From the digest of levantetraose, a hexaoligofructan and a smaller amt. of a pentaoligofructan, but no fructose were sepd., indicating enzymic intermol. levanbiosyl and fructosyl transfer reactions.

=> d 18 ibib ab 30-35

L8 ANSWER 30 OF 35 LIFESCI COPYRIGHT 2003 CSA

ACCESSION NUMBER: 89:92984 LIFESCI

TITLE: Production of a non-reducing fructotrisaccharide from
levan in the culture of *Arthrobacter ureafaciens*.

AUTHOR: Tanaka, K.

CORPORATE SOURCE: Dep. Nutr., Koshien Univ., Takarazuka, Hyogo 665, Japan

SOURCE: AGRIC. BIOL. CHEM., (1989) vol. 53, no. 8, pp. 2275-2276.

DOCUMENT TYPE: Journal

FILE SEGMENT: J

LANGUAGE: English

AB Formation of a non-reducing fructotrisaccharide, designated La sugar, from
levan by an action of the **levan fructotransferase** of *Arthrobacter ureafaciens* has been
reported. This paper reports its **isolation** and identification.

L8 ANSWER 31 OF 35 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1989-00538 BIOTECHDS

TITLE: A method for preparing inulin-**fructotransferase**;
by culturing *Pseudomonas fluorescens*

PATENT ASSIGNEE: Maruzen-Synth.Chem.

PATENT INFO: JP 38219372 B 13 Sep 1988

APPLICATION INFO: JP 1987-52991 10 Mar 1987

PRIORITY INFO: JP 1987-52991 10 Mar 1987

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 1988-297730 [42]

AB A new method for producing inulin-**fructotransferase**
(EC-2.4.1.93) comprises culturing *Pseudomonas* spp., which show enzyme
production, in a culture medium containing fructose polymer and
recovering the enzyme from the culture broth. Inulin-
fructotransferase hydrolyzes inulin to di-D-fructosylfuranose-
1,2':2,3'-dianhydride, which is a sweetener. The enzyme is produced in
higher amounts by *Pseudomonas* spp. than by *Arthrobacter ureafaciens*
and is produced in a more pure form, free from
beta-D-fructofuranosidase (EC-3.2.1.26). The produced enzyme is stable
and can be stored for several mth in a refrigerator. The preferred
bacterium is *Pseudomonas fluorescens* strain MZ No.949 (FERM P-9235) and
is **isolated** from soil. (3pp)

L8 ANSWER 32 OF 35 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1989-03534 BIOTECHDS

TITLE: **Purification** and some properties of inulin-
fructotransferase (depolymerizing) from *Arthrobacter ilicis*;

isolation and characterization

AUTHOR: Kawamura M; Takahashi S; Uchiyama T

LOCATION: Department of Biology, Osaka Kyoiku University, Tennoji-ku,
Osaka 543, Japan.

SOURCE: Agric.Biol.Chem.; (1988) 52, 12, 3209-10

CODEN: ABCHA6

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Inulin-**fructotransferase** (depolymerizing) (EC-2.4.1.93) was
produced by *Arthrobacter ilicis* OKU17B, a soil **isolate**, when
grown on inulin culture medium. The enzyme was **purified** from
culture supernatant by ionexchange on DEAE-Toyopearl 650M and
SP-Toyopearl 650M (twice). The enzyme was **purified** 97-fold, and
14% recovery was achieved. The mol.wt. of the inulin-
fructotransferase was 50,000, comprising 2 subunits of mol.wt.
27,000. The enzyme had maximum activity at pH 5.5 and 60 deg, and was
stable at pH 4-11 and up to 70 deg after incubation at pH 7.0 for 30 min.

Enzyme activity was little affected by various cations at 1 mM (CaCl₂, NiCl₂, ZnCl₂, FeCl₃, CuCl₂, MnCl₂, MgCl₂, CoCl₂ and HgCl₂), differing from the *Arthrobacter ureafaciens* inulin-fructotransferase which was inhibited by 1 mM HgCl₂ and CuCl₂. The principal reaction product after incubation of depolymerizing inulin-fructotransferase with inulin was identified as di-D-fructose-2', 1:2, 3'-dianhydride. The dimeric form of the enzyme differed from inulin-fructotransferases of other spp., such as *Arthrobacter grobiformis* C11-1, where the enzyme was monomeric. (10 ref)

L8 ANSWER 33 OF 35 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 12
 ACCESSION NUMBER: 1989:3410 CAPLUS
 DOCUMENT NUMBER: 110:3410
 TITLE: Enzymic formation of di-D-fructose anhydrides from fructan
 AUTHOR(S): Uchiyama, Takao; Tanaka, Kuniiji; Kawamura, Mishio
 CORPORATE SOURCE: Dep. Biol., Osaka Kyoiku Univ., Osaka, 543, Japan
 SOURCE: Denpun Kagaku (1988), 35(2), 113-20
 CODEN: DPNKAV; ISSN: 0021-5406
 DOCUMENT TYPE: Journal
 LANGUAGE: Japanese

AB The action of a novel inulin (I)-degrading enzyme, inulin fructotransferase (depolyng.) (EC 2.4.1.93), produced by a strain of *Arthrobacter ureafaciens*, is described. The enzyme acts on I to form difructose anhydride (di-O-D-fructofuranose-.alpha.-2,3';.beta.-2;1-dianhydride) (II). II was hydrolyzed by an intracellular enzyme from *A. ureafaciens* and *Klebsiella oxytoca* at .alpha.-2,3'-links to form inulobiose (1-O-.beta.-D-fructofuranosyl-D-fructofuranose) (III). This enzyme was an .alpha.-fructofuranosidase of a new type. It was purified 172-fold from bacterial cells, and had a pH optimum at 7.0 and a mol. wt. of 70,000; it is a dimer consisting of 2 subunits of mol. wt. 36,000. A .beta.-fructofuranosidase which cleaved III was also partially purified from *A. ureafaciens*. Pathways of I metab. through difructose dianhydrides were discussed.

L8 ANSWER 34 OF 35 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 13
 ACCESSION NUMBER: 1985:500785 CAPLUS
 DOCUMENT NUMBER: 103:100785
 TITLE: Intermolecular fructosyl and levanbiosyl transfers by levan fructotransferase of *Arthrobacter ureafaciens*
 AUTHOR(S): Tanaka, Kuniiji; Karigane, Takashi; Fujii, Sachie; Chinzaka, Takuma; Nagamura, Shinichi
 CORPORATE SOURCE: Dep. Biol., Osaka Kyoiku Univ., Osaka, 543, Japan
 SOURCE: Journal of Biochemistry (Tokyo, Japan) (1985), 97(6), 1679-88
 CODEN: JOBIAO; ISSN: 0021-924X
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB A purified levan fructotransferase prepn. from the culture of *A. ureafaciens*, which produces di-D-fructose 2,6':6,2' dianhydride (difructose anhydride IV) from levan by an intramol. levan fructosyl transfer (ILFT) reaction, produced a trioligofructan and a tetraoligofructan from levan in the presence of levanbiose, indicating intermol. fructosyl and levanbiosyl transfer (LFT and LBT, resp.) reactions. The tri- and tetraoligofructans were levantriose and -tetraose, resp. An increase in the levanbiose concn. brought about an increased prodn. of both oligofructans with decreased formation of difructose anhydride IV, supporting the previous theory that the ILFT, LFT, and LBT reactions are catalyzed by the same enzyme. In addn., there existed a roughly stoichiometric relation between the increase and decrease in the prodn. of these oligofructans, and the LBT reaction occurred more intensively than the LFT reaction. The acceptor specificity of the LFT and LBT reactions was studied by using 15

sugars, including mono-, di-, and trisaccharides. The enzyme showed both of the reactions only with levanbiose, -triose, and kestose, indicating that the exposed nonreducing levanbiosyl residue was essential for the acceptor and suggesting the existence of a levanbiosyl acceptor site on the enzyme mol.

L8 ANSWER 35 OF 35 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 14
ACCESSION NUMBER: 1984:63893 CAPLUS
DOCUMENT NUMBER: 100:63893
TITLE: Action of **levan fructotransferase**
of *Arthrobacter ureafaciens* on
levanooligosaccharides
AUTHOR(S): Tanaka, Kuniji; Karigane, Takashi; Yamaguchi, Fumio;
Nishikawa, Shigeko; Yoshida, Noriko
CORPORATE SOURCE: Dep. Biol., Osaka Kyoiku Univ., Osaka, 543, Japan
SOURCE: Journal of Biochemistry (Tokyo, Japan) (1983), 94(5),
1569-78
CODEN: JOBIAO; ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Levan fructotransferase (I) of *A. ureafaciens***
, which produces di-D-fructose 2,6':6,2' dianhydride (difructose anhydride IV) from **levan** by an intramol. transfructosylation reaction, was **purified** to give a single protein band of pI 4.5-4.7 on isoelec. focusing. I had a mol. wt. of 128,000 by gel filtration on Sephadex G-200 and 60,000 on SDS-polyacrylamide disc gel electrophoresis, suggesting that the enzyme is composed of 2 identical subunits. The shortest levanooligosaccharide chain required for difructose anhydride IV formation was detd. to be tetraose. TLC of the enzymic digest of a modified levanhexaose derived from levanhexaose by the redn. of the reducing end to an alditol residue with NaBH₄ gave the difructose anhydride IV spot, suggesting that I attacks the modified levanhexaose mol. from the direction of the nonreducing fructose end. Enzymic digests of levantetraose, -pentaose, and -hexaose gave, in addn. to the difructose anhydride IV spot, spots of oligofructans of lower mobility than the original substrate on TLC. From the digest of levantetraose, a hexaoligofructan and a smaller amt. of a pentaoligofructan, but no fructose were sepd., indicating enzymic intermol. levanbiosyl and fructosyl transfer reactions.

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L2: Entry 1 of 10

File: USPT

Jan 15, 1991

US-PAT-NO: 4985365

DOCUMENT-IDENTIFIER: US 4985365 A

TITLE: Process for producing optically active benzyl alcohol compound

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 2. Document ID: JP 2002017366 A

L2: Entry 2 of 10

File: JPAB

Jan 22, 2002

PUB-NO: JP02002017366A

DOCUMENT-IDENTIFIER: JP 2002017366 A

TITLE: NEW LEVAN FRUCTOTRANSFERASE

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 3. Document ID: JP 11069978 A

L2: Entry 3 of 10

File: JPAB

Mar 16, 1999

PUB-NO: JP411069978A

DOCUMENT-IDENTIFIER: JP 11069978 A

TITLE: LEVAN FRUCTOTRANSFERASE GENE

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Clip Img	Image
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☒ 4. Document ID: JP 01091793 A

L2: Entry 4 of 10

File: JPAB

Apr 11, 1989

PUB-NO: JP401091793A

DOCUMENT-IDENTIFIER: JP 01091793 A

TITLE: PRODUCTION OF DI-D-FRUCTOSYLFURANOSE 2,6':6,2'-DIANHYDRIDE

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Clip Img	Image
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☐ 5. Document ID: JP 01091777 A

L2: Entry 5 of 10

File: JPAB

Apr 11, 1989

PUB-NO: JP401091777A
DOCUMENT-IDENTIFIER: JP 01091777 A
TITLE: PRODUCTION OF LEVAN FRUCTOTRANSFERASE

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 6. Document ID: JP 2002017366 A

L2: Entry 6 of 10

File: DWPI

Jan 22, 2002

DERWENT-ACC-NO: 2002-287313
DERWENT-WEEK: 200233
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TITLE: A new levan fructotransferase

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 7. Document ID: JP 2003512045 W WO 200129185 A1 AU 200114197 A KR
2001037651 A EP 1141236 A1 CN 1340097 A

L2: Entry 7 of 10

File: DWPI

Apr 2, 2003

DERWENT-ACC-NO: 2001-308483
DERWENT-WEEK: 200325
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TITLE: Producing difructose dianhydride IV from sucrose, involves reacting sugar solution in the presence of levansucrase to produce levan, and reacting levan solution in the presence of levan fructotransferase to produce DFA IV

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC	Draw Desc	Image
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☐ 8. Document ID: JP 11069978 A

L2: Entry 8 of 10

File: DWPI

Mar 16, 1999

DERWENT-ACC-NO: 1999-247463
DERWENT-WEEK: 199921
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TITLE: Levan fructotransferase gene - for recombinant production of levan fructotransferase

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC	Draw Desc	Image
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☐ 9. Document ID: JP 01091793 A JP 2614460 B2

L2: Entry 9 of 10

File: DWPI

Apr 11, 1989

DERWENT-ACC-NO: 1989-148148
DERWENT-WEEK: 199726
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TITLE: Di:D-fructosyl-furanose-2,6',6,2'-di: anhydride prepn. - by treating levan with levan fructo-transferase produced by bacterial strain of pseudomonas, for low calorific non-tooth-decaying sugar

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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KWIC	Draw Desc	Image
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☐ 10. Document ID: JP 01091777 A JP 2561100 B2

L2: Entry 10 of 10

File: DWPI

Apr 11, 1989

DERWENT-ACC-NO: 1989-148137

DERWENT-WEEK: 199702

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TITLE: Levan fructo-transferase prepn. - by culturing Pseudomonas bacteria in culture medium contg. fructose polymer, and recovering prod.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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L1 same (levan or mobilis or ureafaciens of difructose dianhydride)	10

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*DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ*L2 L1 same (levan or mobilis or ureafaciens of difructose dianhydride)

10

L2L1 fructotransferase

29

L1

END OF SEARCH HISTORY